Eur J Vasc Endovasc Surg (2009) **38**, 316–322

REVIEW

Homocysteine and Peripheral Arterial Disease: Systematic Review and Meta-analysis

N. Khandanpour [a,*], Y.K. Loke [b], F.J. Meyer [a], B. Jennings [b], M.P. Armon [a]

[a] *Norfolk and Norwich University Hospital NHS Trust, Vascular Surgery Unit, Norwich NR4 7UY, UK*
[b] *University of East Anglia, School of Medicine and Health Policy, Norwich NR4 7TJ, UK*

Submitted 13 November 2008; accepted 9 May 2009
Available online 27 June 2009

KEYWORDS
Homocysteine;
Folic acid;
Intermittent claudication;
Peripheral arterial diseases;
Atherosclerosis;
Homocysteine-lowering therapy

Abstract *Objective:* To evaluate homocysteine (Hcy) levels in patients with peripheral arterial disease (PAD) as compared to unaffected controls, and to review the clinical effects of therapy aimed at lowering homocysteine in PAD patients.
Methods: MEDLINE, EMBASE and Cochrane databases were searched from 1950 to December 2007. We selected observational studies and trials that evaluated Hcy levels in patients with PAD compared to unaffected controls. We also included trials on the effect of Hcy-lowering therapy (folate supplementation) in PAD patients. Continuous outcomes were pooled in a random effects meta-analysis of the weighted mean difference between comparator groups.
Results: We retrieved 33 potentially suitable articles from our search. Meta-analysis of 14 relevant studies showed that Hcy was significantly elevated (pooled mean difference +4.31 μmol l; 95% C.I. 1.71, 6.31, $p < 0.0001$ with significant heterogeneity) in patients with PAD compared to controls. As all 14 studies consistently demonstrated raised plasma Hcy levels in PAD patients, the significant heterogeneity in this meta-analysis probably arises from differences in the degree of Hcy elevation.

The effect of folate supplementation on PAD was tested in eight clinical trials but clinically important end points were inconsistently reported.
Conclusion: Patients with PAD have significantly higher Hcy levels than unaffected controls. However, we did not find any robust evidence on clinically beneficial effects of folate supplementation in PAD.

More than three decades ago, McCully proposed an association between raised plasma total homocysteine (tHcy) levels and the development of atherosclerosis.[1] This correlation has led many groups to investigate the role of tHcy-lowering therapy in the prevention and treatment of

* Corresponding author.
E-mail address: nkh950@yahoo.com (N. Khandanpour).

1078-5884/$36
doi:10.1016/j.ejvs.2009.05.007

atherosclerosis. One of the manifestations of systemic atherosclerosis is peripheral arterial disease (PAD), a common condition that affects about 27 million individuals in North America and Europe.[2]

In 1995, Boushey et al. reviewed 27 studies on Hcy and atherosclerotic disease and performed a meta-analysis of five studies demonstrating an association between elevated plasma Hcy levels and PAD.[3] Many new observational and experimental studies on Hcy and PAD have since been published. Recent clinical trials in atherosclerotic patients have also focussed on the potentially beneficial effects of folate supplementation in tackling raised plasma Hcy levels.

We aimed to update Boushey's analysis by conducting a systematic review to assess Hcy levels in patients with PAD as compared to unaffected controls. We also looked for trials that evaluated the beneficial effects of Hcy-lowering therapy (folate supplementation) in such patients.

Methods

The literature was systematically reviewed and synthesised according to the methods below.

Search strategy

The following databases were searched: MEDLINE (January 1950—December 2007), EMBASE (January 1974—December 2007) and Cochrane (January 1950—December 2007), restricted to English-language articles only. The Medical Subject Headings (MeSH terms) such as 'intermittent claudication', 'peripheral vascular disease', 'leg', 'femoral artery', 'popliteal artery' or 'lower extremity', 'folic acid', 'homofolic acid', 'vitamin B complex' and the text words 'folic acid' and 'folate' and 'homocysteine' were used. Additional 'related articles' suggested by PubMed, reference lists of the retrieved articles and reviews on the subject were also manually evaluated to identify any other relevant published articles.

Eligibility criteria

Studies were included in the observational meta-analysis of Hcy association with PAD if the plasma Hcy levels of cases and controls were reported, and we were able to estimate the standard deviation.

Studies were considered for inclusion in the meta-analysis of controlled clinical trials if PAD patients had been treated with single or combined therapy with folic acid, vitamin B6 or B12 as compared to no specific intervention. Eligible trials had to recruit PAD patients defined by ankle brachial pressure index (ABPI) less than 0.9, clinical evidence of intermittent claudication or clearly diminished foot pulses plus obstruction of one major peripheral artery on angiography.

Exclusion criteria

Studies were excluded if the literature was not in English or they did not have a control group. Previous meta-analyses and case reports were not included in this meta-analysis.

Study selection was performed by three independent reviewers (NKH, FJM and MPA), and any discrepancies were resolved through discussion with other reviewers (BJ or YKL).

Data extraction and synthesis

Baseline characteristics and results from the included studies were recorded onto a spreadsheet. We extracted data depending on whether the main variable investigated was Hcy levels, or if the study was focussed on the effect of folate administration.

Where relevant, RevMan 5.014 (Nordic Cochrane Centre) was used in a meta-analysis to calculate the pooled odds ratio (OR) for dichotomous outcomes, and the weighted mean difference (WMD) for continuous outcomes. Statistical heterogeneity was assessed using the Cochran Q, and the I^2 statistics. I^2 values of 50% or more indicate a substantial level of heterogeneity. If the heterogeneity was substantial ($I^2 > 50\%$), a random effects meta-analysis was performed; otherwise a fixed effect model was used as default.

Results

A total of 214 potentially relevant studies were retrieved from the electronic databases.

Association of plasma tHcy with PAD

After reviewing the abstracts on the effect of Hcy on PAD, 179 of the 214 studies were deemed unsuitable. From the studies scrutinised completely, 19 were excluded for the following reasons (see Fig. 1):

1. Coronary, cerebral and peripheral artery diseases were not separated (two studies).[4,5]
2. No report of Hcy levels for the PAD and control cohorts (three studies).[6–8]
3. No suitable PAD cohort or no control group (five studies).[9–13]

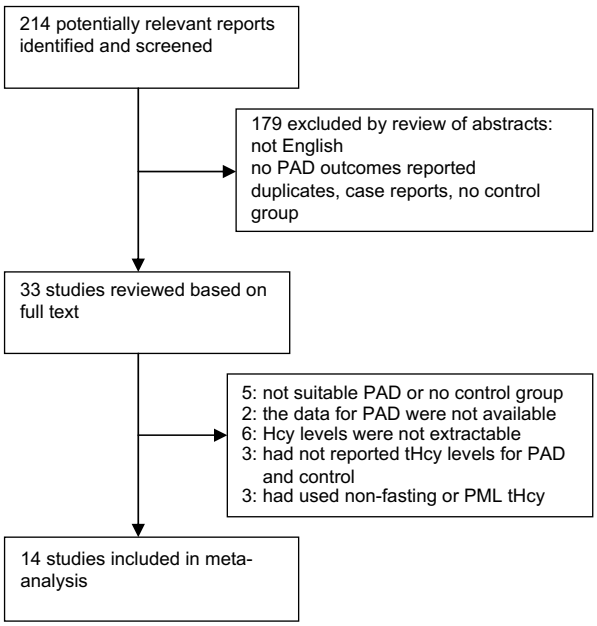

Figure 1 A flow diagram of the study selection for meta-analysis on association of plasma tHcy levels and the risk of development of PAD.

Table 1 Study design and baseline characteristics of observational studies on the association of hyperhomocysteinaemia and PAD

Author	Country	Number of participants		Men *n* (%)		Age		Diabetes mellitus %		Total cholesterol[a] (mmol/L)	
		Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Malinow et al.[23]	US	103	47	26 (55.3%)	18 (38.3%)	70.1 ± 10.6	65.9 ± 3.9	21 (65.6%)	1 (2.1%)	5.4 ± 1.4	6.2 + 1.1
Taylor et al.[24]	US	214	103	110 (51%)	53 (51.5%)	64 ± 13.1	NR	NR	NR	NR	NR
Molgaard et al.[25]	Sweden	78	98	78 (100%)	98 (100%)	Range: 45–69	Range: 45–69	9%	0%	5.73 ± 1.04	5.46 ± 0.92
Bergmark et al.[26]	Sweden and Norway	58	58	29 (50%)	29 (50%)	49	Age matched	NR	NR	NR	NR
Mansoor et al.[27]	Sweden	65	65	35 (53.8%)	34 (52.3%)	*m* = 47 *M* = 50[b]	Age matched	7 (10.8.8%)	1 (1.5%)	*m*: 6.3, *f*: 6.6	*m*: 6.1, *f*: 5.8
Cheng et al.[28]	Hong Kong	100	100	64 (64%)	64 (64%)	67	67	35 (35%)	9 (9%)	5.88 ± 1.63	5.44 ± 1.04
Valentine et al.[29]	US	50	45	95 (100%)	45(100%)	46 ± 0.5	45 ± 0.5	11 (22%)	5 (11%)	18 (36%)[a]	9 (20%)[a]
Erren et al.[30]	Germany	15	62	11 (73%)	56 (90.3%)	63 ± 9	56 ± 13	9 (60%)	9 (15%)	5 (32%)[a]	9 (15%)[a]
Bunout et al.[31]	Chile	32	24	22 (68.8%)	12 (50%)	69.6 ± 11	69.6 ± 11	0%	0%	5.62 ± 1.21	5.6 ± 1.2
Rassoul et al.[32]	Germany	85	51	85 (100%)	51 (100%)	60 ± 11	Age matched	NR	NR	5.62 ± 1.5	5.3 ± 0.7
Stricker et al.[33]	Switzerland	51	51	32 (62.7%)	32 (62.7%)	68.3	68.5	18 (32.3%)	0	5.64 ± 1.04	6.26 ± 1.19
Loncar et al.[34]	Germany	40	40	28 (70%)	8 (30%)	51.8 ± 7.5	51.8 ± 7.5	0%	0%	5.8 ± 1.23	5.7 ± 1.21
Darius et al.[35]	Germany	1230	5650	109 (19.8%)	2373 (42%)	Mean age overall: 72.5 ± 5.3		Overall number with diabetes: 1741 (25.3%)		Overall number with lipid disorder: 3557 (51.7%)	
Bhargava et al.[36]	India	244	205	NR	NR	NR	NR	NR	NR	NR	NR

m: male, f: female, NR: not reported.
[a] Percentages represent hypercholesterolaemia.
[b] Values represent median.

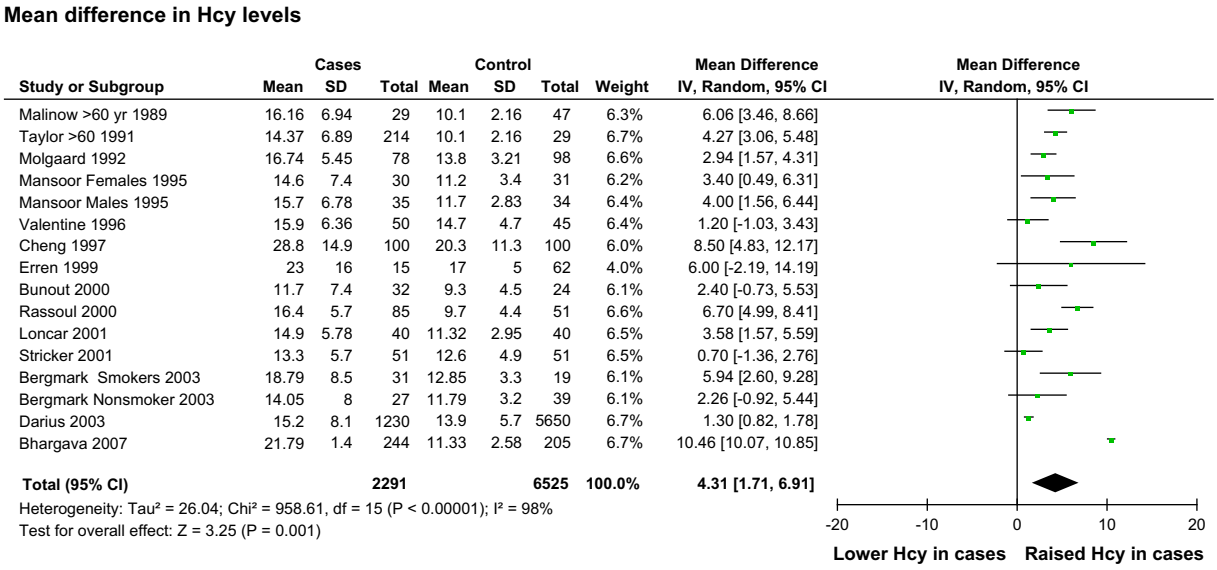

Figure 2 Meta-analysis of observational studies on association of Hcy levels and PAD. Mean plasma tHcy for PAD in individuals was compared with healthy individuals. A random effects (inverse variance) model was used to calculate the summary mean difference.

4. Hcy levels were not extractable for meta-analysis (six studies).[14–19]
5. Used non-fasting or post-methionine loading Hcy levels (three studies).[20–22]

Table 1 summarises the study design and baseline characteristics of the studies included. Fourteen studies were analysed using a random effects meta-analysis (Fig. 2).

This meta-analysis of more than 3000 patients in 14 cross-sectional and prospective studies showed that patients with PAD had, on average, Hcy levels that were 4.31 μmol l^{-1} higher than those of controls without PAD. The elevated Hcy levels were a consistent finding in all 14 studies, although the magnitude or size of the difference varied considerably, ranging from an increase of 0.70 up to 10.46 compared to controls. This diversity most likely accounts for the significant heterogeneity amongst the studies, with the Cochran Q of $P < 0.001$, and I^2 of 98% despite the direction of effect being entirely consistent. Thus, a random effects model was used to incorporate inter-study variation, and arrived at an 'average' estimate of effect, rather than the single 'true' estimate obtained with the fixed effects model. Removing any single study from the meta-analysis did not substantially alter the direction or magnitude of the summary statistic.

Clinical trials assessing the effect of Hcy-lowering therapy (folate supplementation) on PAD

Owing to the diverse outcomes reported, we were unable to conduct a meta-analysis on major clinical end points such as the need for amputation.

There were a few studies which have used progression of PAD as a major end point. Table 2 summarises the study design, characteristics and outcomes of these studies. One randomised controlled trial (RCT) assessed the effect of 150 μg folic acid day^{-1} on patients with PAD.[39] After 12 months intervention, the mean ABPI in these patients improved from 0.47 to 0.53. Pain-free walking distance, which was measured by using treadmill set at 3 km h^{-1} and 10% slope, also improved by 280 m. Patients' walking distance before the onset of claudication increased in the intervention group ($p < 0.001$); ABPI also increased ($p < 0.05$). In this study, however, other nutritional supplements were also used and none of the patients was prescribed a statin. A similar 6-week intervention study did not demonstrate any improvement in ABPI.[40]

''One study on PAD patients has reported a positive correlation between the inflammatory marker and hs-CRP (hs-CRP, highly sensitive C reactive protein)''.[42] The results of two further clinical trials on the effect of folate on cardiovascular markers such as CRP, inflammatory mediators and cholesterol profiles were not consistent.[41,42] Two trials assessed the effect of folate supplementation on endothelium dilation and found that folate was effective in improving endothelium-dependent dilatation. However, the clinical relevance of the above findings is not known.

Discussion

Our meta-analysis of 14 epidemiological studies shows that patients with PAD had consistently higher levels of Hcy as compared to controls. These data suggest that hyperhomocysteinaemia may either be a marker of PAD, or be aetiologically implicated in the development of PAD. Our results are consistent with the findings of a multicentre case control study that was performed in nine European countries on patients with PAD, cerebrovascular disease and coronary artery disease.[4] The multicentre study found that plasma Hcy concentrations greater than the 80th percentile for control subjects were associated with an increased risk of vascular disease, independent of all traditional risk factors. It has also been shown that plasma tHcy is higher in patients with multi-level PAD when

Table 2 Study design and baseline characteristics of the controlled clinical trials of folate intervention in peripheral arterial disease

Author	Study design and setting	Dose and duration	Participant numbers		Men *n* (%)		Age		DM *n* (%)		Total[a] cholesterol		Fall in plasma Hcy % (μmol/L)	Measured outcomes
			Folate	Controls	Folate	Controls	Folate	Controls	Folate	Controls	Folate	Controls		
Brattström et al.[43]	Non-randomised clinical trial Sweden 1990	10 mg for 4 weeks	37	46	21 (56.8%)	22 (48.9%)	52 ± 6	52 ± 7	0	0	Overall, 35.1% had elevated levels		52.8% (12.2)	Plasma Hcy reduced
Dudman et al.[44]	Non-randomised clinical trial Australia 1993	5 mg for 2 weeks	11	31	9 (50%)	20 (64.5%)	Male: 43.6 ± 12.5; female: 36.5 ± 13.9	Male: (34.6 ± 14.5); female: premenopause 28.8 ± 8.1; post-menopause: 59.1 ± 8.3	NR	NR	NR	NR	49%	Plasma Hcy reduced
Woo et al.[37]	Double blind/RCT Hong Kong 1999	10 mg for 8 weeks	17	17	15 (88%)	15 (88%)	54 ± 10	54 ± 10	0	0	5.7 ± 0.8	5.4 ± 1.0	17.3% (1.7)	Endothelium-dependent dilatation of vessels: 2.5% rise. Oral GTN dependent dilation of vessels: 0.2% increase
Mayer et al.[38,c]	Non-randomised clinical trial Czech 2002	5—10 mg for 3 months	33	26	19 (57.6%)	5 (19.2%)	64.8 ± 3.1	67.3 ± 4.4	6 (18.2%)	0	6.29	NR	25.1% (5.8)	Plasma Hcy reduced
Sydow et al.[40]	Double blind/RCT Germany 2003	10 mg for 8 weeks	9	9	7 (77.8%)	7 (77.8%)	69.3	68.8	1 (11.1%)	1 (11.1%)	5.9	5.8	44.9% (7.1)	ABPI unchanged. Endothelium-dependent dilation of vessels: 0.5% change. Oral GTN dependent dilation of vessels: 1% decrease.
Carrero et al.[39]	Double blind/RCT Spain 2005	150 μg for 12 months	30	30	30 (100%)	30 (100%)	62.4 ± 1.6	65.6 ± 1.7	5 (16%)	6 (20%)	5.43	5.42	15% (2.61)[b]	ABPI increased from 0.46 ± 0.13 to 0.53 ± 0.13. Pain-free walking distance improved from 52.7 to 316.17
Ziegler et al.[41]	Double blind/RCT Austria 2005	5 mg for 6 weeks	27	22	34 (69.3%)	18 (66.7%)	68 ± 7	69 ± 8	9 (33%)	9 (40.9%)	4.7 ± 0.83	158 ± 77	34.2% (6.95)	Plasma Hcy reduced. ADMA, hs-CRP, triglycerides, TC and HDL change
Schernthaner et al.[42]	Double blind/RCT Austria 2006	5 mg for 6 weeks	33	32	24 (72.7%)	22 (68.8%)	68.6 ± 7.6	68 ± 8.1	16 (51.5%)	16 (50%)	5.2 ± 1.2	5.2 ± 1.3	33% (6.3)	Changes in hs-CRP, IL-6, IL-8, IL-18, MCP-1, TFPI, TF and fibrinogen

n: number, DM: diabetes mellitus.
[a] Percentages represent the prevalence of hypercholesterolaemia.
[b] Values represent total participants.
[c] Values represent median.

compared with subjects with either suprainguinal or infrainguinal disease alone.[26] In addition, people with hyperhomocysteinaemia develop PAD at an earlier age.[45] Plasma levels of Hcy are influenced by several factors such as underlying genetic factors, as well as by plasma folate and vitamin B12 levels. This may explain why vascular patients may not always have very high plasma levels of Hcy.

Although we found inconsistent evidence for folate supplementation in PAD, other meta-analyses have examined the effect of folic acid on coronary and cerebral artery diseases. Wang et al. performed a meta-analysis of folic acid supplementation on 16 841 participants from eight clinical trials and concluded that folic acid supplementation reduced the risk of stroke by 18%.[46] A similar meta-analysis, however, reported that the overall effect of folic acid supplementation on coronary artery disease was not significant.[47] This discrepancy might be attributed to the arterial site. In addition, it has been suggested that folate therapy may have a marked beneficial effect as a primary rather than as a secondary intervention.[47]

Our study has some limitations. Firstly, there was substantial heterogeneity amongst the studies, probably because of differences in the magnitude of excess Hcy values rather than any variation in the direction of effect as all the studies reported a consistent association between elevated plasma Hcy concentrations and the risk of PAD. Eleven of the included studies in the meta-analysis showed that hyperhomocysteinaemia had a significant association with an increased risk of developing PAD.

Although we have used a broad search strategy, we have only been able to analyse English studies, and it is possible that we may have missed other studies where authors have not fully reported their findings in published articles. However, all 14 studies in our review have shown a consistent effect (with a highly significant *p*-value of <0.001 and lower limit of 95% confidence interval to be 1.71) and our meta-analysis is based on 8800 patients. It would take several large studies with differing findings to change the results of our meta-analysis.

In comparison to observational data, controlled trials are a more reliable form of scientific evidence since they minimise spurious causality and bias. However, we were unable to identify any well-designed trials suitable for a meta-analysis of the clinical effects of Hcy-lowering therapy. It remains to be verified whether folate intervention would be beneficial in patients with less severe established atherosclerosis and without end organ damage, such as renal failure and myocardial infarction. While more than 20 000 patients have been studied in clinical trials of the effect of Hcy-lowering therapy on coronary and cerebral artery diseases, only 290 patients with PAD have been investigated using important clinical outcomes. A double-blind RCT, assessing the outcomes of folic acid supplementation on clinical signs of PAD, has recently been performed in our centre that may improve our understanding of the role of folate supplementation on PAD.

In conclusion, evidence from our meta-analysis confirms the hypothesis that patients with PAD have significantly higher levels of Hcy. However, we also conclude that there is little published evidence about the effects of folate supplementation on major clinical outcomes relating to PAD.

Conflict of Interest

None declared.

References

1 McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. *Am J Pathol* 1969 Jul;**56**(1):111–28.

2 Belch JJ, Topol EJ, Agnelli G, Bertrand M, Califf RM, Clement DL, et al. Prevention of atherothrombotic disease network. Critical issues in peripheral arterial disease detection and management: a call to action. *Arch Intern Med* 2003 Apr 28; **163**(8):884–92.

3 Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *JAMA* 1995 Oct 4;**274**(13):1049–57.

4 Robinson K, Arheart K, Refsum H, Brattström L, Boers G, Ueland P, et al. Low circulating folate and vitamin B6 concentrations: risk factors for stroke, peripheral vascular disease, and coronary artery disease. European COMAC Group. *Circulation* 1998 Feb 10;**97**(5):437–43.

5 Verhoeff BJ, Trip MD, Prins MH, Kastelein JJ, Reitsma PH. The effect of a common methylenetetrahydrofolate reductase mutation on levels of homocysteine, folate, vitamin B12 and on the risk of premature atherosclerosis. *Atherosclerosis* 1998 Nov;**141**(1):161–6.

6 Wilmink AB, Welch AA, Quick CR, Burns PJ, Hubbard CS, Bradbury AW, et al. Dietary folate and vitamin B6 are independent predictors of peripheral arterial occlusive disease. *J Vasc Surg* 2004 Mar;**39**(3):513–6.

7 Ebly EM, Schaefer JP, Campbell NR, Hogan DB. Folate status, vascular disease and cognition in elderly Canadians. *Age Ageing* 1998 Jul;**27**(4):485–91.

8 Fowkes FG, Lee AJ, Hau CM, Cooke A, Connor JM, Lowe GD. Methylene tetrahydrofolate reductase (MTHFR) and nitric oxide synthase (ecNOS) genes and risks of peripheral arterial disease and coronary heart disease: Edinburgh Artery Study. *Atherosclerosis* 2000 May;**150**(1):179–85.

9 Daskalopoulou SS, Pathmarajah M, Kakkos SK, Daskalopoulos ME, Holloway P, Mikhailidis DP, et al. Association between ankle–brachial index and risk factor profile in patients newly diagnosed with intermittent claudication. *Circ J* 2008 Mar;**72**(3):441–8.

10 Jaar BG, Plantinga LC, Astor BC, Fink NE, Longenecker C, Tracy RP, et al. Novel and traditional cardiovascular risk factors for peripheral arterial disease in incident-dialysis patients. *Adv Chronic Kidney Dis* 2007 Jul;**14**(3):304–13.

11 Khawaja FJ, Bailey KR, Turner ST, Kardia SL, Mosley Jr TH, Kullo IJ. Association of novel risk factors with the ankle brachial index in African American and non-Hispanic white populations. *Mayo Clin Proc* 2007 Jun;**82**(6):709–16.

12 Goessens BM, van der Graaf Y, Olijhoek JK, Visseren FLSMART Study Group. The course of vascular risk factors and the occurrence of vascular events in patients with symptomatic peripheral arterial disease. *J Vasc Surg* 2007 Jan;**45**(1):47–54.

13 van den Berg M, Stehouwer CD, Bierdrager E, Rauwerda JA. Plasma homocysteine and severity of atherosclerosis in young patients with lower-limb atherosclerotic disease. *Arterioscler Thromb Vasc Biol* 1996 Jan;**16**(1):165–71.

14 Spark JI, Laws P, Fitridge R. The incidence of hyperhomocysteinaemia in vascular patients. *Eur J Vasc Endovasc Surg* 2003 Nov;**26**(5):558–61.

15 Sofi F, Lari B, Rogolino A, Marcucci R, Pratesi G, Dorigo W, et al. Thrombophilic risk factors for symptomatic peripheral arterial disease. *J Vasc Surg* 2005 Feb;**41**(2):255–60.

16 Graham IM. Interactions between homocysteinemia and conventional risk factors in vascular diseases. *Eur Heart J* 1994; **15**(Suppl.):530.
17 Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. *JAMA* 2001 May 16;**285**(19):2481—5.
18 Clarke R, Daley L, Robinson K, Naughten E, Cahalane S, Fowler B, et al. Hyperhomocysteinemia: an independent risk factor for vascular disease. *N Engl J Med* 1993;**324**(17):1149—55.
19 Ciccarone E, Di Castelnuovo A, Assanelli D, Archetti S, Ruggeri G, Salcuni N, et alGENDIABE Investigators. Homocysteine levels are associated with the severity of peripheral arterial disease in Type 2 diabetic patients. *J Thromb Haemost* 2004 Jul;**2**(7):1212—3. author reply 1214.
20 van den Bosch MA, Bloemenkamp DG, Mali WP, Kemmeren JM, Tanis BC, Algra A, et al. Hyperhomocysteinemia and risk for peripheral arterial occlusive disease in young women. *J Vasc Surg* 2003 Oct;**38**(4):772—8.
21 Taylor Jr LM, Moneta GL, Sexton GJ, Schuff RA, Porter JM. Prospective blinded study of the relationship between plasma homocysteine and progression of symptomatic peripheral arterial disease. *J Vasc Surg* 1999 Jan;**29**(1):8—19.
22 Bloemenkamp DG, van den Bosch MA, Mali WP, Tanis BC, Rosendaal FR, Kemmeren JM, et al. Novel risk factors for peripheral arterial disease in young women. *Am J Med* 2002 Oct 15;**113**(6):462—7.
23 Malinow MR, Kang SS, Taylor LM, Wong PW, Coull B, Inahara T, et al. Prevalence of hyperhomocyst(e)inemia in patients with peripheral arterial occlusive disease. *Circulation* 1989 Jun; **79**(6):1180—8.
24 Taylor Jr LM, DeFrang RD, Harris Jr EJ, Porter JM. The association of elevated plasma homocyst(e)ine with progression of symptomatic peripheral arterial disease. *J Vasc Surg* 1991 Jan; **13**(1):128—36.
25 Molgaard J, Malinow MR, Lassvik C, Holm AC, Upson B, Olsson AG. Hyperhomocyst(e)inaemia: an independent risk factor for intermittent claudication. *J Intern Med* 1992;**231**(3): 273—9.
26 Bergmark C, Mansoor MA, Swedenborg J, de Faire U, Svardal AM, Ueland PM. Hyperhomocysteinemia in patients operated for lower extremity ischaemia below the age of 50 — effect of smoking and extent of disease. *Eur J Vasc Surg* 1993Jul;**7**(4):391—6.
27 Mansoor MA, Bergmark C, Svardal AM, Lønning PE, Ueland PM. Redox status and protein binding of plasma homocysteine and other aminothiols in patients with early-onset peripheral vascular disease. *Arterioscler Thromb Vasc Biol* 1995 Feb;**15**(2): 232—40.
28 Cheng SW, Ting AC, Wong J. Fasting total plasma homocysteine and atherosclerotic peripheral vascular disease. *Ann Vasc Surg* 1997 May;**11**(3):217—23.
29 Valentine RJ, Kaplan HS, Green R, Jacobsen DW, Myers SI. Cl homocysteine, and hypercoagulable states in young men with premature peripheral atherosclerosis. *J Vasc Surg* 1996 Jan; **23**(1):53—61.
30 Erren M, Reinecke H, Junker R, Fobker M, Schulte H, Schurek JO, et al. Systemic inflammatory parameters in patients with atherosclerosis of the coronary and peripheral arteries. *Arterioscler Thromb Vasc Biol* 1999 Oct;**19**(10): 2355—63.
31 Bunout D, Petermann M, Hirsch S, de la Maza P, Suazo M, Barrera G, et al. Low serum folate but normal homocysteine levels in patients with atherosclerotic vascular disease and matched healthy controls. *Nutrition* 2000 Jun;**16**(6):434—8.
32 Rassoul F, Richter V, Janke C, Purschwitz K, Klötzer B, Geisel J, et al. Plasma homocysteine and lipoprotein profile in patients with peripheral arterial occlusive disease. *Angiology* 2000 Mar; **51**(3):189—96.
33 Stricker H, Soldati G, Balmelli T, Mombelli G. Homocysteine, vitamins and gene mutations in peripheral arterial disease. *Blood Coagul Fibrinolysis* 2001 Sep;**12**(6):469—75.
34 Loncar R, Hrboka V, Tabakovic-Loncar V, Ostojic Z, Deussen A. Screening of plasma homocysteine in peripheral arterial disease. *Ann Med* 2001 Feb;**33**(1):48—54.
35 Darius H, Pittrow D, Haberl R, Trampisch HJ, Schuster A, Lange S, et al. Are elevated homocysteine plasma levels related to peripheral arterial disease? Results from a cross-sectional study of 6880 primary care patients. *Eur J Clin Invest* 2003 Sep; **33**(9):751—7.
36 Bhargava S, Parakh R, Manocha A, Ali A, Srivastava LM. Prevalence of hyperhomocysteinemia in vascular disease: comparative study of thrombotic venous disease vis-à-vis occlusive arterial disease. *Vascular* 2007 May—Jun;**15**(3):149—53.
37 Woo KS, Chook P, Lolin YI, Sanderson JE, Metreweli C, Celermajer DS. Folic acid improves arterial endothelial function in adults with hyperhomocystinemia. *J Am Coll Cardiol* 1999 Dec;**34**(7):2002—6.
38 Mayer O, Filipovský J, Hromádka M, Svobodová V, Racek J, Mayer Jr O, et al. Treatment of hyperhomocysteinemia with folic acid: effects on homocysteine levels, coagulation status, and oxidative stress markers. *J Cardiovasc Pharmacol* 2002 Jun; **39**(6):851—7.
39 Carrero JJ, López-Huertas E, Salmerón LM, Baró L, Ros E. Daily supplementation with ($n-3$) PUFAs, oleic acid, folic acid, and vitamins B-6 and E increases pain-free walking distance and improves risk factors in men with peripheral vascular disease. *J Nutr* 2005 Jun;**135**(6):1393—9.
40 Sydow K, Schwedhelm E, Arakawa N, Bode-Böger SM, Tsikas D, Hornig B, et al. ADMA and oxidative stress are responsible for endothelial dysfunction in hyperhomocyst(e)inemia: effects of L-arginine and B vitamins. *Cardiovasc Res* 2003 Jan;**57**(1): 244—52.
41 Ziegler S, Mittermayer F, Plank C, Minar E, Wolzt M, Schernthaner GH. Homocyst(e)ine-lowering therapy does not affect plasma asymmetrical dimethylarginine concentrations in patients with peripheral artery disease. *J Clin Endocrinol Metab* 2005 Apr;**90**(4):2175—8.
42 Schernthaner GH, Plank C, Minar E, Bieglmayer C, Koppensteiner R, Schernthaner G. No effect of homocysteine-lowering therapy on vascular inflammation and haemostasis in peripheral arterial occlusive disease. *Eur J Clin Invest* 2006 May;**36**(5):333—9.
43 Brattström L, Israelsson B, Norrving B, Bergqvist D, Thörne J, Hultberg B, et al. Impaired homocysteine metabolism in early-onset cerebral and peripheral occlusive arterial disease. Effects of pyridoxine and folic acid treatment. *Atherosclerosis* 1990 Feb;**81**(1):51—60.
44 Dudman NP, Wilcken DE, Wang J, Lynch JF, Macey D, Lundberg P. Disordered methionine/homocysteine metabolism in premature vascular disease. Its occurrence, cofactor therapy, and enzymology. *Arterioscler Thromb* 1993 Sep;**13**(9): 1253—60.
45 Fryer RH, Wilson BD, Gubler DB, Fitzgerald LA, Rodgers GM. Homocysteine, a risk factor for premature vascular disease and thrombosis, induces tissue factor activity in endothelial cells. *Arterioscler Thromb* 1993 Sep;**13**(9):1327—33.
46 Wang X, Qin X, Demirtas H, Li J, Mao G, Huo Y, et al. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. *Lancet* 2007 Jun 2;**369**(9576):1876—82.
47 Bazzano LA, Reynolds K, Holder KN, He J. Effect of folic acid supplementation on risk of cardiovascular diseases: a meta-analysis of randomised controlled trials. *JAMA* 2006 Dec 13; **296**(22):2720—6.